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The Composition of Brain Lecithin

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THE COMPOSITION OF BRAIN LECITHIN

BY

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A.B. University of Illinois, 1913

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1915

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

June 4, 1915

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Juanita Elizabeth Darrah

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DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

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Final Examination

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THE COMPOSITION OF BRAIN LECITHIN.

I. INTRODUCTION.

Definition and Classification.

Lecithin belongs to that class of organic compounds designated as phosphatides, a subdivision of the lipoids. The phosphatides, in some respects, resemble fats but they differ from the latter in that they contain nitrogen and phosphorus in various proportions. Bang (1) defines lipoids as those "cell compounds which are soluble in organic solvents such as ether, chloroform and benzene." Apparently the phosphatides are found in all cells and are necessary for the life of the cell. Our present knowledge of the phosphatides has been attained largely through the systematic study of the composition of the brain (2). One of the earliest attempts to isolate a "phospholupine" was made by Couerbe in 1834. He obtained from the brain a substance, "cerebrote", which contained phosphorus and was the forerunner of the protagones described later by others (3). The first system of classification of phosphatides, carefully worked out, was published by Thudicum in 1875 (4). He presented a more complete scheme of classification in 1901, as follows (5):

A. Group of phosphorus-containing constituents which he designated as phosphatides.

1. Monophosphatides N:P = 1:1
   (Monamino-Monophosphatides)
   a. Lecithin.
   b. Kephalin.
   c. Paramyelin.

        d. Myelin.
    2. Monophosphatides N:P = 2:1
        a. Amidomyelin, etc.
    3. Diphosphatides N:P = 2:2
        a. Assurin.
    4. Nitrogen-containing Phosphatide-sulphatides.
    5. Nitrogen-free Monophosphatides.
B. Nitrogen-containing Phosphorus-free Group.
    1. Cerebrosides.
    2. Cerebrin acids.
    3. Cerbrosulphatides, etc.
C. Constituents containing only three elements.
D. Organoplastic or Albuminous Substances.
E. Inorganic Principles.
        (acids, bases, salts).

Bang (2) makes use of a somewhat different classification by which the phosphatides containing an unsaturated acid form one class, and those containing only saturated fatty acid radicals form another. The members of the unsaturated group are semi-solid or viscous; they do not crystallize; they oxidize in the air; they are easily decomposed and react readily with other substances. For these reasons they are particularly difficult to obtain pure (2). Lecithin is a monamino-monophosphatide having the characteristics of the unsaturated group.

Occurrence.

Lecithin and its compounds are very abundant and widely distributed in nature. Lecithin has been prepared not only from brain tissue (4) (5), spinal cord and nerve tissue, but also from

muscle (7), heart (8), liver, kidneys (9), blood (1), bone-marrow (10 milk (11), egg yolk (12-16), and other materials of animal origin. It has likewise been prepared from various vegetable sources, such as seeds (17) (18) of lupinus lutens and albus (19), vicia sativa, pinus cembra and picea excelsa; from wheat meal, oat meal (20), and from the green leaves of aesculus hippocastanum. Because of the difficulty in obtaining pure lecithin, there has been much disagreement concerning the identity of these preparations. Yoshimoto as recently as 1910 made this statement, "Lecithin-Chemie noch nicht so weit geklärt ist, dasz es möglich wäre, Fütterungs versuche mit zuverlässig reinen und einheitlichen Phosphatiden anzustellen."

II. Importance.

That the question of the preparation and composition of pure lecithin is an important one is readily conceded by all who realize the vital significance of lipoids in general and of lecithin in particular. There is much disagreement concerning the essential part which lipoid foods play in maintaining life. Some investigators (Stepp--22) have found that mice die when fed on food completely freed from lipoids by extraction with alcohol and ether. Stepp (23) has shown that a mixture of lipoids consisting of lecithin, cholesterol, kephalin, cerebron and phytin will not replace the substance removed by alcohol-ether extraction of bread and milk, for feeding mice. Recent investigations raise the question whether the superiority of organic phosphorus compounds may not be dependent upon minute quantities of certain compounds commonly associated with them, "vitamines", etc. However, this question may be settled, the result is to put a new emphasis on the value of the natural organic food stuff as compared with synthetic nutrients (24). In many experiments, there

has been no guarantee of the purity of the organic compounds fed, and no particular effort to guard against the influence of other useful compounds associated with the organic phosphorus in the natural products and also in the isolated compounds as used in these investigations (24). For a positive solution of this problem, more definite knowledge is required concerning all the compounds involved, especially the lipoids.

On one point all seem to agree, i. e.,-- if lipoids are not necessary for maintenance of life, at any rate they exert a very beneficial influence, in many ways, upon life processes. Bang (25) says that if our knowledge of pure chemistry of lipoids is still unsatisfactory and incomplete, the same is true of the biochemistry of the lipoids in greater degree. He considers the action of cell lipoids to be important in secretion, excretion, and taking up nourishment by the cell, especially where the material concerned must react with the lipoid membrane. "Also the other life reactions of the cell are likewise intimately connected with the lipoids. It is likewise no mere coincidence that the most highly organized cells are always richest in lipoids."

Changes in salt content of the blood may alter the permeability of the plasma membrane, thus influencing osmosis. The hydrophilic character of lecithin may allow this compound to carry into the cell, substances to which it might not otherwise be permeable. The anions having decreasing power to precipitate lecithin stand in this order:--

$SO4 > C2H5O_2 > Fl > Cl > NO_3 > Br > I > CnS$ (26)

Fe and Al possess the most active precipitation powers of the trivalent cations. Koch (27) suggests that the emulsions formed by lecithins may be precipitated by divalent cations unless prevented by

uni- and tri-valent cations, and this may furnish an explanation of the changes brought about by electrolytes in the living cell, "Lecithins", Koch says, (1) "may through colloidal solution, furnish the basis for bringing about the necessary viscosity by the facility with which they are influenced by Na and Ca ions. Production of viscosity may have a bearing on the significance of lipoids in the red blood cells." (2) "They share in metabolism of the cell, and by their unsaturated acids and methyl groups bound to nitrogen, take part in reactions as yet unexplained."

Glikin (28) has shown that those animals born helpless contain more lecithin in their spinal cords than those active and self-sustaining from birth. Lecithin content increases in percentage, with age. Bergell (29) concluded that in all cells lecithin may be regarded as necessary to cell cleavage. Loew (30) considers the chief function of lecithin to be participation in respiration; it is the form into which fats must be changed to become combustible in the protoplasm. According to this theory, by the transformation of fatty matter into lecithin, the higher fatty acids are offered to the protoplasm in a soluble form. After one molecule of acid is oxidized, other molecules may enter the place of the former. Thus the same molecules of glycerophosphoric acid may serve repeatedly as vehicles for oxidation of molecules of fatty acids. That digestion of fat is accelerated by prescence of lecithin is affirmed by Usuki (31). He found that presence of lecithin in the stomach favored the saponification of the neutral fats.

On the contrary, Kalaboukoff and Terroine (32) found lecithin had no activating power in pancreatic digestion except at high concentrations in the case of olive oil. Terroine obtained negative results with respect to the lipolytic action. In hydrolysis of starch

digestion of casein, or pancreatic coagulation of milk, lecithin seemed to have no accelerating effect.

A storage function of lecithin has been noted by several investigators. Mesernizky (33) has determined the effect of incubation on the lecithin content of eggs. The lecithin of the yolk is a storage of food for the developing germ. It is used in development of the skeletal system; the phosphoric acid group in building phosphoproteins, and lecithin as a whole for liberation of energy through oxidation of the fat radical.

There is abundant evidence that lecithin improves the general state of nutrition. Desgrez and Zaky (34) mention the favorable influence exercised by lecithin of egg on nutritive changes. Their analyses showed that lecithin caused a constant retention of phosphorus. From experiments on guinea pigs, rabbits and dogs, they conclude that the H_5PO_4 retained by the organism is normally utilized for the development of the cells of the bones and of the nerves. They note an increase in lecithin content of the nervous tissue after feeding of lecithin. Slowtzoff (35) 1906, confirmed these results by feeding experiments upon humans with regard to phosphorus retention and notes an increased nitrogen retention and decrease in urinary sulphur. He interprets these results as indicating a promotion by lecithin, of protein synthesis. Cronheim and more recently Patta (36) recommend lecithin for adults as well as children, especially for replacement of lost nitrogenous material. In harmony with this is the work of Yoshimoto (21). His experiments indicate that when lecithin is fed to dogs one-half of the phosphorus remains in the body as lecithin or in some other form. Franchini has noted lecithin retention in liver, to a less extent in muscles, but not at all in brain tissue. Other experiments indicative of similar results have been carried on by Des-

selkin (37). A recent review of the literature on this subject closes with the following summary:- "Animals receiving lecithin, especially if the basal ration be low in phosphorus, and the animals in a state of depleted reserves, show increased appetite and gain in live weight. Phosphorus retention is favored; there has been observed an increase of ash and of phosphorus in the skeleton, of phosphorus in the muscles, and of phosphorus and lecithin in the brain; fasting animals live longer if they receive lecithin and their livers and muscles are increased in lecithin content by ingestion of lecithin" (38).

Pathological.

From a consideration of the above results, dieticians have recommended lecithin-rich foods such as milk and eggs for improving state of nutrition. Lecithin has been indicated by physicians in certain pathological derangements. The medicinal value of butter-milk depends upon its content of lecithin which is higher than that of ordinary milk. Certain wines contain considerable lecithin (Dornic) (39). The bouquet of wines and nutritional value as well, is believed by Paturel (40) to depend upon the lecithin content.

In paralysis, Glikin (41) has noted the disappearance of or decrease in amount of lecithin normally present. In syphilis, the toxin combines with lecithin and is excreted in conjugated form through the intestinal mucosa. A lowering of the lecithin content of tissues results and is responsible for such derived disorders as tabes and paralysis. An abnormally high content of lecithin in the sera and feces of such patients was accompanied by an abnormally low content in the bone-marrow (Peritz--42). Glikin (43) has called attention to the abnormally high phosphorus content of the blood of patients suffering with polycythaemia rubra, indicating a lecithin content of blood

above normal.

As to lecithin -therapy there is still some doubt. Serono (44) found it had a tonic effect acting more quickly than arsenic. There resulted gain in appetite, improvement in general condition, and rapid increase of red blood corpuscles. Ruggia (44) reports lecithin has a nutritive and curative value when injected in cases of anaemia and infantile athrepsia. Morichan (44) Beauchant concludes: (1) Lecithin stimulates the appetite, and leads to rapid gain in weight. It stimulates growth and increases the number of red blood corpuscles.
(2) It increases urea, total nitrogen elimination and the utilization of nitrogen, and diminishes uric acid.
(3) In tuberculosis lecithin is valuable in early stages as an auxiliary. It may be of similar value in diabetes by improving general conditions. It also gives good results in anaemias, Cachexias and convalescence. In neuresthenia it is especially valuable.

Aries (44) considers lecithin a valuable remedy in all cases where "denutrition" is to be combatted, especially in old age. Maillon found ingestion of lecithin beneficial; in chlorosis it caused a constant increase in body weight. Snowman (44) gives clinical reports of benefit derived from use of sanatogen, i. e., improved blood conditions in chlorosis, chorea, and tuberculosis. Milkowicz (44) found in vitro that lecithin caused a great increase of phagocytosis of Staphylococcus and tubercle bacilli. Bain regards lecithin chiefly as a metabolic stimulus, its effects on the nervous system being secondary to improvement in general state of nutrition. On the other hand Koch (44) believes there is no good scientific basis for clinical administration of lecithin to the adult. He considers phosphorus preparations used as drugs are much less effective for supplying the requirements for growth of the brain than are phosphorus-rich foods.

Bang(25) has shown that lipoids have several important functions in the cells, i. e., they play a part in fermentation reactions.

1. It is possible that lipoids act as peculiar enzymes.
2. They may act as enzyme activators.
3. They may occur as Kinases.
4. They may control enzyme formation, a rolle analogous to that of active immunizing agent.
5. They may serve to retard enzyme actions.

With respect to toxic activity, lipoids may have several different functions.

1. They may act as poisons.
2. Lipoids may show activating action toward lysines.
3. Lipoids may bear to lysines the same relation that kinases bear to enzymes.
4. Lipoids appear to be inhibitors.
    a. toward toxines.
    b. toward lysines.
5. Lipoids may bring about a lysine formation--i. e., action of lipoids as antigens.
    a. Cobra venom as well as bee and scorpion poison contain an hemolysin which is activated by lecithin (25).

Arrhenius (45) mentions the marked influence which lecithin has upon hemolysis by acids. Hemolytic action becomes equal to that caused by four times the quantity of acid, by addition of a small amount of lecithin. Vay (46) found that lecithin emulsions of 1%-10% have the property of agglutinizing pathogenic bacilli after a few hours, and altering their staining properties. Lecithin was found to be of value in resisting toxins.--Ferking (47). After treating guinea

pigs with subtoxic doses of typhus-lecithin emulsion the animals are protected against a several-fold toxic dose.

Several investigators have pointed out the rolle of lecithin in narcosis. In the case of strychnine, Koch (48) says, "The central nervous system, especially the cord, by its high phosphatide content, is enabled to pick the strychnine out of the blood stream on account of the affinity of the lecithin and kephalin for the strychnine. Strychnine probably enters into combination with lecithin through some relation to its unsaturated fatty acid group." "There is some evidence that $CH\ Cl_3$ as distinguished from pure ether has power to form a combination with lecithin, a phenomenon which may be brought into relation with its slow elimination and consequent tendency to produce delayed poisoning."

From studies on nicotine de Waele (49) concludes that the action of a lethal dose of an alkaloid is decreased or inhibited by addition of an equimolecular amount of lecithin. Lipoids promote entrance of water soluble substances into the cell protoplasm. They likewise promote entrance of aqueous alkaloid solutions into nerve cells. The alkaloids gradually unite with the nerve cells. Removal of the poison may be brought about by simultaneous introduction of lecithin into the organism with the alkaloid. Clinical experience confirms this.

Reiss (50) determined that lecithin with chloroform is able to take up the ferments rennet and trypsin, as neither constituent alone can. This may have some significance with reference to the role of lecithin in the body. It is likened to the activating power of lecithin toward hemolysis of cobra poison.

Since we have now briefly noted the physiological significance of lecithin, we may understand why the preparation and chemical

composition of so important a compound merits most thorough investigation. We shall now pass to a brief historical sketch of previous work on this subject.

III. HISTORICAL.

Lecithin was discovered by Gobley (2) in 1846, in egg yolk. He studied the lecithin of brain tissue in 1847. Liebreich (5) prepared lecithin from brain, in 1865. The latter used a method typical of the earlier ones. The blood-free and finely chopped brain was shaken with water and ether. The mixture was allowed to separate into two layers at 0°. A large part of the cholesterol and traces of protagon were taken up by the ether. Both solutions were filtered. The mass remaining on the filter was taken up in 85% alcohol at 45°C. and filtered. On cooling to 0°C. a precipitate settled out. This was filtered off and washed free from cholesterol with cold ether. The dried mass is then dissolved in alcohol at 45°C., filtered and allowed to crystallize. We now understand this protagon to be a mixture instead of a single compound. Diakonow considered protagon as a mixture of cerebrin and lecithin. In 1879 Gamgee (51) and Blankenhorn investigated protagon and concluded it to be a mixture.

Strecker (13) in 1868, prepared lecithin from egg yolk by extracting with alcohol and ether, removing the ether, adding alcohol as long as turbidity resulted, then precipitation with platinic chloride. Strecker also precipitated lecithin from ether solutions by use of cadmium chloride. The metals were removed by H_2S, thus leaving the lecithin chloride. The free lecithin was obtained by treating the chloride with silver oxide.

Lecithin was also prepared by Diakonow (52) in 1868. He ex-

tracted beef brain with ether. Then the remaining mass was extracted with absolute alcohol at 40°C. When the alcoholic extract was cooled to 0°C. a precipitate separated. After extraction of this precipitate with ether, the protagon remained as a residue. This was repeatedly extracted with ether at room temperature. The residue from the last treatment was dissolved in absolute alcohol. On cooling this solution to -7° a white substance separated which had the properties of lecithin.

Baumstark (53) in 1885 called attention to the fact that earlier investigators have extracted the brain material at high temperatures and with strong alcohol, both of which, he thought, would tend to decompose the original compound. He says, "One must as nearly as possible maintain body temperature, especially for the first extraction of the material from the tissue." He declares that only Liebreich and Blankenhorn and Gamgee have mentioned this fact before.

Gilson (54) in 1888 studied lecithin prepared from egg yolk by the method of Diakonow with a slight modification. The modification consisted in increasing the yield by distilling off the ether from the ether extract, then dissolving in petroleum ether. The petroleum ether solution was filtered and shaken repeatedly with 75% alcohol in a separatory funnel. The alcohol solutions drawn off were united, filtered, and the petroleum ether was removed by distillation. After allowing the solution to stand several days in a cool place, lecithin and some cholesterol settled out. The cholesterol was removed by successive ether and alcohol treatments.

Zuelzer (55) was one of the first to suggest that acetone seemed to be a suitable medium for removing cholesterol from lecithin. In 1899, he found that acetone divided his ether extract almost quantitatively into two groups, the ether acetone solution containing

cholesterol, and the cholesterol-free residue. The lecithin was precipitated from a somewhat concentrated ether solution, almost free from cholesterol. Other phosphorus containing substances were found in the precipitate. These were removed by dissolving in ether, protagon remained insoluble. On adding excess of alcohol to the ether solution, part is precipitated (probably kephalin) and lecithin remains in the solution.

Koch (56) described his method in 1902. The finely ground brain tissue was freed from water and extractives by eight hours heating with acetone. The acetone was filtered off and the material dried by gentle heat was extracted 2--3 days with cold ether. "The ether filtrates were combined and allowed to evaporate gradually in tall cylinders. A fine white precipitate of unknown nature (probably protagon) settled; the concentrated ether solution was drawn off by a pipette. On addition of alcohol to this solution, the kephalin separated as a white precipitate. After evaporation of the alcohol-ether solution, the lecithin residue was freed from cholesterol by acetone and recrystallized from acetic ester."

Erlandsen (8) (3) cast discredit upon Thudicum's preparation of lecithin. In 1906, he called attention to Thudicum's methods. The latter were of two sorts.

First-- Precipitation of extracts of brain with alcoholic cadmium chloride, and separation of the precipitate by solvents.

Second-- Precipitation of extracts of brain with alcoholic lead acetate and ammonia.

Erlandsen questioned the use of cadmium chloride. He found lecithin was not precipitated quantitatively from heart tissue, by Cd Cl_2. One-third of the lecithin remained in solution. He further considered that Cd Cl_2 decomposed lecithin. The prolonged treatment with boiling

ether and benzene as practiced by Thudicum was considered as likely to cause decomposition. Erlandsen also objected to the method of boiling with ammonia and lead acetate and prolonged dialysis for removal of $Cd\ Cl_2$.

Erlandsen prepared lecithin from egg yolk and from heart and striated muscle. The material was dried on glass plates before a fan at 40° C. Then it was successively extracted with ether and alcohol at room temperature or at 40° C. with a stream of CO_2 passing through it. The residue from this extract was partly soluble in water-free ether. This solution gave a precipitate with acetone, part of which was soluble in cold absolute alcohol and part insoluble. The soluble part consisted of lecithin. In 1907 Bang (57) confirmed Erlandsen's work. He stated that all previous methods of preparation of lecithin had involved its partial decomposition. The only method considered reliable by Bang was that of Erlandsen.

Stern and Thierfelder (58) in 1907, dried their material, egg yolk, as Erlandsen had done. The material was taken up in ether, solution filtered, concentrated and precipitated with acetone. This treatment was repeated until only a slight precipitate was thrown down by acetone. Air and light were excluded. Solvents were anhydrous and freshly distilled. No heat was used except to evaporate the alcohol, which was done in vacuo at body temperature. The "white substance" was removed by repeated centrifuging of the ether solution. The lecithin was kneaded up with acetone to remove fat and cholesterol. The ether and alcohol soluble part of this lecithin residue contained kephalin which was removed by centrifuging the alcohol solution.

In 1909, Fränkel (14) described the preparation of lecithin as follows:- Extract brain tissue with hot alcohol. On cooling, white material separates. Extract this residue with ether, and precipitate

the kephaline with absolute alcohol. Myelin can be precipitated by alcoholic ammoniacal lead acetate solution. Concentrate the filtrate and precipitate lecithin by excess alcoholic cadmium chloride. Erlandsen's objections would apply to this method.

The same year MacLean (59) prepared lecithin from heart tissue. His method was essentially that of Erlandsen. The material precipitated from ether extracts by acetone contained cuorin, "white substance", fat, and cholesterol. The white substance was removed by centrifuging. The precipitation from ether by means of acetone was repeated until all white substance, fat, and cholesterol had been removed. From the ether solution of the resulting material, cuorin was precipitated by four volumes of absolute alcohol. The lecithin remained in solution.

In 1913, Trier and Eppler (60), each made important investigations on lecithin. The latter prepared his lecithin from egg yolk by the method of Stern and Thierfelder. He confirmed Erlandsen's theory that $Cd\ Cl_2$ decomposes lecithin. The lecithin was shown to be poorer in carbon, hydrogen and oxygen by use of $Cd\ Cl_2$. Trier (61) studied both egg yolk lecithin and lecithin from plant seeds, especially beans and oat grits. He made a comparative study of extraction methods on oat grits. The plant seed lecithins were found to contain reducing sugar. The highest per cent of reducing sugar was obtained in the extraction with alcohol by gently warming. The largest yield of lecithin was obtained by extraction of the fat-free oat-grits with benzene and methyl alcohol.

Properties of Lecithin (57).

Lecithin is usually described as a white or orange colored,

waxy substance, soluble in ether, chloroform, and alcohol. In water it swells and gradually passes into an opalescent, colloidal solution. It is also soluble in carbon disulphide, benzene, and methyl alcohol but nearly insoluble in cold acetone and methyl acetate. Lecithin is very hygroscopic. It is also readily oxidized by the air and is unstable, especially if exposed to light in moist condition.

Lecithin absorbs iodine because of the unsaturated fatty acid which it contains. Different lecithins give different iodine values ranging from 38%-110%. Mayer (62) found lecithin to have an optical rotation $(a)_D = + 11.04^\circ$. He believed that by enzymes an asymmetric action is shown, the r-lecithin disappears and leaves l-lecithin. He believes that most lecithin preparations are racemic.

Lecithin is very active chemically. There are two types of reaction which are common to all lecithins.

1. They add substances in molecular proportion to form fairly well characterized compounds.

2. Form compounds whose nature is uncertain. Whether these are of purely chemical nature or adsorption compounds is not known. Lecithin combines with acids and bases and forms addition compounds with salts of the heavy metals. The compounds with the chlorides of mercury, cadmium, gold and platinum are especially important.

Bang (57) gave the following summary of the classes of organic compounds with which lecithin combines:

1. Glucosides
2. Alkaloids
3. Toxins
4. Cholesterol
5. Enzymes.
6. Dyes
7. Carbohydrates
8. Proteins.

These substances have not been studied.

Constitution of Lecithin

A.- Elementary Analyses. ([illegible])

AUTHOR	DATE	SOURCE	C	H	N	P	FORMULA
Thudicum	?	Brain	66.75	18.67	1.81	4.00	$C_{43}H_{84}NPO_9$
Koch	1902	"	64.05	10.40	1.80	3.79	---------------
Erlandsen	1906	Heart	66.29	10.17	1.87	3.95	$C_{43}H_{80}NPO_9$
MacLean	1908	"	66.27	10.32	1.85	3.975	---------------
Erlandsen	1907	Muscle of thigh	65.96	10.20	1.82	3.95	$C_{43}H_{80}NPO_9$
Stern and Thierfelder	1907	Egg-yolk	64.63	10.96	2.08	3.97	---------------
MacLean	1909	Egg-yolk	64.18	10.60	1.88	3.95	---------------
Baskoff	1908	Liver	64.64	10.71	1.95	4	---------------

B.- Structure.

1. Hydrolysis Methods.

It has been pretty well established that lecithin is the choline ester of α, β, di-glyceride of phosphoric acid. This has been determined by the investigations of Diakonow, Strecker, Ulpiani, and Willstätter (63) and Lüdecke using analytical methods; and by Hundeshagen and also Grün and Kade using synthetic methods. The analytical demonstrations have been based chiefly upon hydrolyses. About the earliest hydrolysis of lecithin was done by Liebreich in 1865 (5), using aqueous barium hydroxide. From the protagon thus hydrolysed, he obtained in the filtrate the barium salt of glycero-phosphoric acid and a base which he designated as neurin. The barium salts of the fatty acids, stearic and one unsaturated acid which he said was not oleic remained insoluble and were removed with the $Ba\,CO_3$ on removing the excess barium with CO_2.

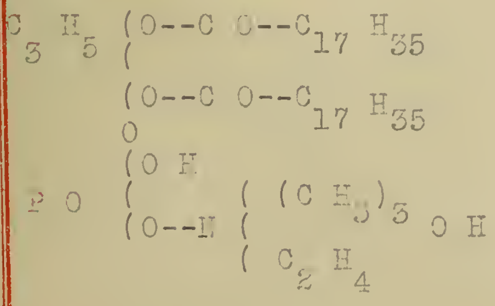
In 1868, Diakonow (12) hydrolysed lecithin by dilute H_2SO_4 and also $Ba(OH)_2$. He considered lecithin as a salt compound of neurin (tri-ethyl oxethyl ammonium) with di-stearyl glycero-phosphoric acid, the latter being decomposed by $Ba(OH)_2$ into stearic acid and glycero-phosphoric acid salts of barium. He set forth the following equation:

$$\underset{\text{lecithin}}{C_{44}H_{90}NPO_9} + 3H_2O = \underset{\text{neurin}}{C_5H_{15}NO_2} + 2(\underset{\text{stearic acid}}{C_{18}H_{36}O_2}) + \underset{\text{glycero-phosphoric}}{C_3H_9PO_6}$$

In 1849 Strecker discovered the compound, choline. In 1868 Strecker (13) hydrolysed lecithin with $Ba(OH)_2$ and obtained as hydrolysis products choline and barium salts of oleic, margaric and glycero-phosphoric acids. He considered lecithin as capable of having the properties of a base, of an acid and of a fat, simultaneously. By adding $Pt\,Cl_6$ to an alcoholic lecithin solution Strecker obtained a precipitate insoluble in alcohol, soluble in ether and chloroform; the lecithin behaves like a base. If it were a salt, one should obtain neurin-Pt Cl_6, insoluble in alcohol, ether, and $CHCl_3$. For this reason, he inferred that lecithin is an ether compound, that neurin is bound to the distearin-glycero-phosphoric acid by the O of the O H group.

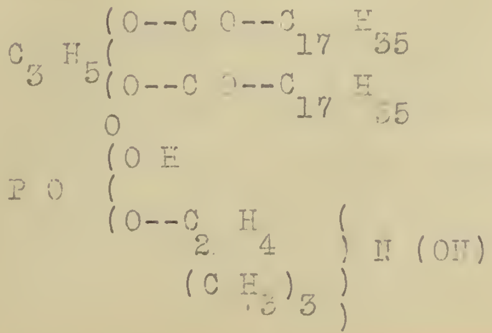
The structural formulae of Diakonow and Strecker were thus expressed:-

Diakonow



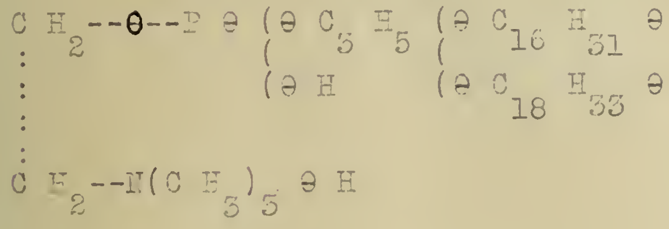
$$C_3H_5\left(\begin{array}{l}O{-}{-}C\ O{-}{-}C_{17}H_{35}\\ O{-}{-}C\ O{-}{-}C_{17}H_{35}\\ O\end{array}\right.\qquad PO\left(\begin{array}{l}O\,H\\ O{-}{-}N\left(\begin{array}{l}(C\,H_3)_3\quad O\,H\\ C_2\,H_4\end{array}\right.\end{array}\right.$$

Strecker



$$C_3H_5\left(\begin{array}{l}O{-}{-}C\ O{-}{-}C_{17}H_{35}\\ O{-}{-}C\ O{-}{-}C_{17}H_{35}\\ O\end{array}\right.\qquad PO\left(\begin{array}{l}O\,H\\ O{-}{-}C_2\,H_4\\ \quad(C\,H_3)_3\end{array}\right)N\,(OH)$$

The structural formula of lecithin in which were contained margaric and oleic acids was indicated by Strecker:-



$$\begin{array}{l} CH_2\text{--}O\text{--}P\,O\left(\begin{array}{l}O\,C_3H_5\left(\begin{array}{l}O\,C_{16}H_{31}\,O\\ O\,C_{18}H_{33}\,O\end{array}\right.\\ O\,H\end{array}\right.\\ \vdots \\ CH_2\text{--}N(CH_3)_3\,O\,H\end{array}$$

Strecker demonstrated the structure of choline by Wurtz synthesis heating ethylene oxide with trimethyl-amine-hydrochloride. Hundeshagen in 1883 was able to synthesize a compound isometric with lecithin. He concluded that lecithin could not be a salt but in agreement with Strecker's view must be an ether.

Diakonow based his argument that lecithin is a salt on two facts.

1. By Ba $(OH)_2$ hydrolysis neurin and barium salts of glycero-phosphoric acid and of phosphoric acid.

2. By dilute H_2SO_4, lecithin was rapidly decomposed with formation of neurin sulphate and free distearin-glycero-phosphoric acid.

In opposition to these arguments Gilson (54) found in 1888 that (1st) action of Ba $(OH)_2$ is equally well explained by considering lecithin as an ether.

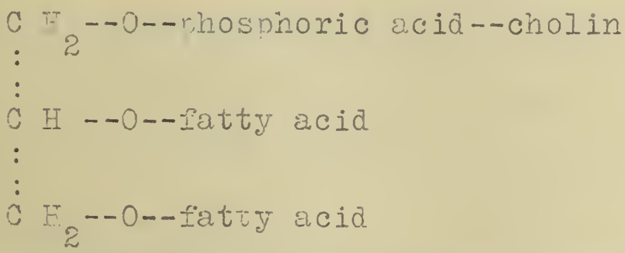
(2nd) He found that dilute H_2SO_4 did not act rapidly, as would be the case if lecithin were a salt compound. The gradual and incomplete action of H_2SO_4 indicated to Gilson that hydrolysis proceeds by first addition of water to the lecithin, by which neurin and distearin-glycero-phosphoric acid results. There is then a subsequent formation of free H_3PO_4, glycero-phosphoric acid and fatty acids.

Gilson's explanation of the acid hydrolysis as involving

hydration was previously shown by Diakonow who heated lecithin with water in a tube to 120°C, thus obtaining the same hydrolysis products as yielded by acid hydrolysis. Gilson also found that a 1% solution of Na OH caused complete hydrolysis into neurin and salts of glycerophosphoric acid and of fatty acids. He argued that if lecithin were a neurin salt, then very dilute Na OH should set free the neurin before it completely decomposed the lecithin. As this was not the case, he felt justified in declaring that lecithin is not a salt but an ether.

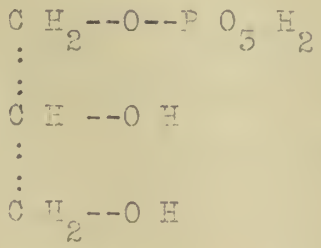
Ulpiani (63) proved definitely that lecithin from hen's eggs is dextro-rotatory. On this basis he gave the unsymmetrical formula I the preference.

I.



$$\begin{array}{l} CH_2\text{--O--phosphoric acid--cholin} \\ \vdots \\ CH\text{ --O--fatty acid} \\ \vdots \\ CH_2\text{--O--fatty acid} \end{array}$$

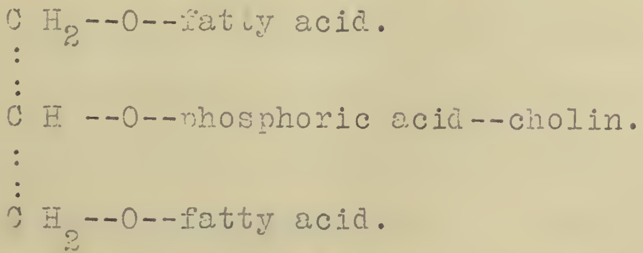
By hydrolysis with $Ba(OH)_2$, Willstätter and Lüdecke confirmed this formula. They found the optically active glycero-phosphoric acid to have the formula,--



$$\begin{array}{l} CH_2\text{--O--}PO_3H_2 \\ \vdots \\ CH\text{ --O}H \\ \vdots \\ CH_2\text{--O}H \end{array}$$

Thus the unsymmetrical structure II was precluded.

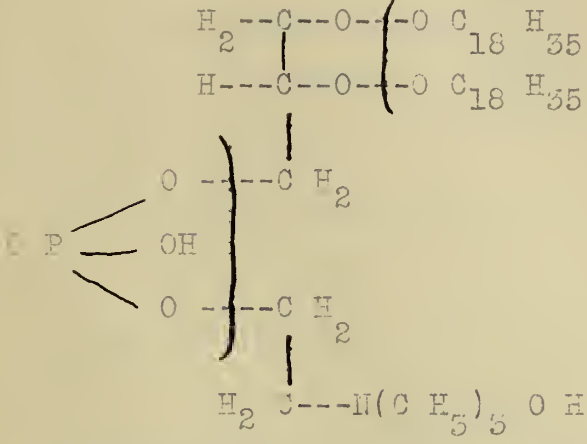
II.



$$\begin{array}{l} CH_2\text{--O--fatty acid.} \\ \vdots \\ CH\text{ --O--phosphoric acid--cholin.} \\ \vdots \\ CH_2\text{--O--fatty acid.} \end{array}$$

Malengreau (65) and Prigent performed some hydrolysis experiments in 1912, using various concentrations of different acids,

noting the influence of the time of continuance of the hydrolysis and the speed of the hydrolysis under these varied conditions. According to the usual formula set forth for lecithin,



the splitting off of the different radicals in the molecule seem to begin at the same time, and the appearance of H_3PO_4 at the same time with the fatty acids seems probable. The splitting off of the latter, however, takes place very much more rapidly than the hydrolysis of the glycero-H_3PO_4 ester. The choline does not behave like an ester of H_3PO_4. It is split off rapidly and holds equal pace with the fatty acid hydrolysis. It seems to the last named investigators to be impossible to consider lecithin as a cholin ester of phosphoric acid. They declare the nature of the combination to be still unknown. Thus they would refute the theory of Strecker and Gilson. They decided the entire soluble nitrogen to be in the form of cholin.

MacLean (59) (66) used Ba $(OH)_2$ in ethyl and methyl alcohols, dilute H_2SO_4 and dilute H Cl as hydrolysing agents. For examining the basic products he very much preferred dilute H Cl hydrolysis. In each case, he obtained a choline nitrogen value which did not account for the theoretical yield of cholin calculated on the basis of total nitrogen.

Cholin Values.

heart lecithin = 42% of total N_2.
egg lecithin = 66% " " "
trade lecithin = 80% " " "

After complete hydrolysis, a residue which contained from 5-9% "residual nitrogen" was obtained.

(2) Hydrolysis Products.

a). Bases.

After hydrolysis, MacLean (66) filtered off the resulting fatty acids. The filtrates were evaporated to dryness and the residue taken up in absolute alcohol and the alcoholic solution was filtered and platinum chloride was added to the filtrate. The platinum double salt contained 31.7% Pt. The cholin platinum chloride failed to completely account for the total nitrogen of the lecithin. By use of $Cd\ Cl_2$, instead of platinum chloride, he was able to obtain 75% of the total nitrogen as choline nitrogen. He concluded that the formula of lecithin was still incompletely determined. He found no experimental basis for supposing that cholin is partially decomposed during hydrolysis. He was able to recover quantitatively the choline from a mixture of all the possible hydrolysis products of lecithin.

These facts may seem to indicate the presence of another nitrogen-containing base not precipitable by $Cd\ Cl_2$. Trier (18) (61) isolated amino ethyl alcohol from the hydrolysis products of lecithin from beans, oat grits, and eggs. He prepared the gold salt by treatment of the water solution of the chloride with strong $H\ Cl$ and gold chloride solution. On long standing in a dessicator beautiful crystals over a centimeter long were obtained. The gold determination gave 49.29% gold. Calculated 49.17% for amino ethyl alcohol chlor-

aurate.

$$\begin{array}{l} CH_2\text{--}NH_2\text{--}HCl\ AuCl_3 \\ \vdots \\ CH_2OH \quad \text{melting point } 188^{\circ}\text{--}190^{\circ}. \end{array}$$

Amino ethyl alcohol was also characterized by the picrolonate derivative which according to Knorr (71) has the formula $C_2H_7NO\text{--}C_{10}H_8N_4O_5$ with 21.54% nitrogen and melts with dark coloration and decomposition with rapid heating at 225°C, with slow heating at 220°C. Eppler (60) later confirmed Trier's work when he identified amino ethyl alcohol as a product of hydrolysis of lecithin from egg yolk.

Trier (67) was able to synthesize cholin by methylation of amino ethyl alcohol. Trier supposed that in building up lecithin in life processes, the "conjugated phosphoric acid first combines with glycol to form a di-glyceride phosphoric acid glycol ester, or nitrogen-free lecithin. This is then changed by action of ammonia to an amino lecithin (cholin lecithin) which in a physiological way undergoes methylation." That amino ethyl alcohol is not produced from cholin during hydrolysis was proved by Baumann (68) who was unable to obtain any increase in amino nitrogen by Van Slyke method, after boiling choline with H_2SO_4. There seems to be some evidence that amino ethyl alcohol may be a basic constituent of at least certain of the lecithins.

b). Acids.

Erlandsen found 65-70% of lecithin consisted of fatty acids. Identification of the acid constituents has caused much dispute. Thudicum considered oleic acid to be characteristic of lecithins, and that some other acid is usually present; such as palmitic or margaric. Liebreich found stearic acid and Stecker obtained margaric

acid. Henriques (69) and Hansen showed that there must be some acid present more unsaturated than oleic. Cousin (70) claimed discovery of linoleic acid in lecithin. Erlandsen also considered linoleic acid as a constituent of lecithin.

After hydrolysis with alkali, the acids remain in solution as salts. On acidifying the solution with H Cl or acetic acid, the free acids are precipitated, with the exception of glycero-phosphoric acid, which remains in solution. Trier precipitated the choline by phosphotungstic acid, removed the excess of the latter by $Ba(OH)_2$. The excess barium was then removed by saturation with CO_2. On addition of alcohol and concentration of the solution, barium glycerophosphate was precipitated.

Henriques and Hansen used the following method:- The acids were purified by repeated washing with hot water, were dissolved in ether and shaken with water in a separatory funnel. The ether was removed in vacuum. During the whole procedure, a stream of CO_2 was passed through the solutions to prevent oxidation of unsaturated acids. For separation of the liquid from the solid acids, the lead soaps were prepared and separated by dissolving the soaps of the liquid acids in ether. The iodine values obtained on the total fatty acids and on the liquid fatty acids in each case were higher than could be given by oleic acid alone. They consider linoleic acid to be present.

Cousin obtained an iodine value for total fatty acids of egg lecithin of 51-7% which indicates 60-40% of oleic acid. He prepared the barium soaps of the liquid fatty acids and fractionated them with a benzene-alcohol mixture. He thought linoleic acid was present. By oxidation of the unsaturated acids with permanganate, he obtained dioxystearic and tetroxystearic acids. Cousin also found 30-40%

of stearic acid and 60-70% palmitic acid in the saturated acids.

EXPERIMENTAL.

The object of this work was to determine the structure of brain lecithin, first to find out whether it contains amino ethyl alcohol as Trier et al., have found for certain lecithins; then to attempt a solution of the problem concerning the acid constituents.

IV Preparation of Lecithin.

Sheep and beef brains were received in fresh condition directly from the packing house still frozen and were placed at once into the ice box. After carefully removing the blood clots and any loose surface membrane, the brains were finely ground in a food grinder. They were then placed in a suitable dehydrating solution. For this purpose, acetone was preferred. After standing two days, the acetone was filtered off and thoroughly pressed out of the tissue in a fruit press. For this purpose portions of the tissue were tied up in clean cloth. The material was then placed in fresh acetone and this process and renewal of acetone were repeated at least three times. The second and third extractions removed most of the fat and some extractives such as cholesterol.

The dehydrated material was put into a good grade of acetone in a well-stoppered bottle until such a time as convenient to work it up. Portions of this tissue were filtered out, dried on glass plates in a current of air and placed in double volumes of benzene. The benzene was agitated in contact with the tissue on a shaking machine. Part of the extractions were made hot by allowing a stream of water heated

to 65°C to flow over the bottle containing the mixture, while it was shaking. The extraction period was from two to four hours. The benzene solution was filtered off and the tissue freed from benzene so far as possible by the fruit press. A second benzene extraction was then made.

In a few cases an attempt was made to remove more lecithin from the tissue by a hot alcohol extraction, following the benzene extraction. The amount of product thus secured was inappreciable. A few trials were made using ether extraction instead of benzene. This solvent seemed much less satisfactory than benzene and is more likely to cause oxidation of the lecithin.

The benzene extracts were concentrated almost to dryness by distilling under reduced pressure. Distillation was done on the steam bath in a carbon dioxide atmosphere. The light brown residue was dissolved in the smallest possible volume of ether which had been freshly distilled over calcium chloride. This solution was found to contain kephalin, lecithin and "white substance". The kephalin was precipitated by adding a volume of absolute alcohol equal to two and one-half times the volume of the ether solution. As thus precipitated, the kephalin is very light brown in color and is in waxy condition, easily pressed together.

Any suspended kephalin was filtered out, and the alcohol ether filtrate was evaporated with reduced pressure in a carbon dioxide atmosphere. During all such distillation periods the temperature did not rise over 45°C. When evaporated almost to dryness, the residue was dissolved in a small portion of redistilled ether and by addition of a double volume of acetone, the lecithin together with some "white material" was precipitated. The precipitate is yellow or very light brown and adheres to the walls of the precipitation jar

in waxy masses. After filtering off the ether-acetone solution, the precipitate was thoroughly pressed and worked together with fresh acetone, in much the same manner in which butter is worked to remove the buttermilk.

As rapidly as possible, this precipitate which was still impure lecithin , was freed from acetone and placed in a vacuum dessicator. The dessicator was wrapped in cloth to exclude light, and allowed to evacuate for several hours. Sometimes the dessicators contained H_2SO_4 and sometimes $CaCl_2$ as dehydrating agent. When thoroughly dry, the material was dissolved in freshly distilled ether and placed in tall stoppered cylinders. It was allowed to stand in the ether over night. The "white substance" settled out and the clear supernatant solution was syphoned off. This solution was tested for kephalin, by addition of alcohol. Usually kephalin was present and the alcohol treatment and subsequent distillation had to be repeated.

After reprecipitation of the lecithin by the above method, the ether treatment was also repeated to remove any "white substance." The original benzene extract seems to contain, in addition to lecithin and kephalin, a protagon which has not been thoroughly disintegrated until the alcohol ether treatment has been repeated several times. It is therefore necessary to repeat the test for kephalin at least three times. As a criterion of purity of lecithin we used:

1. Absence of kephalin
2. No deposition of "white substance" when allowed to stand in ether solution.

In the latter part of the work, it was found that the lecithin was precipitated in more compact form, by adding the ether solution of the material to the acetone instead of by the reverse method. The last purification of the lecithin was made by dissolving it in

petroleum ether, then adding this solution gradually to double its volume of acetone. It was necessary to centrifuge this solution to entirely remove the lecithin therefrom. Six samples of lecithin were prepared as follows:

Lecithin Preparations.

Source	Wt. of Product.
I. Beef (Old material re-purified)	5.0000 g.
II. Beef Brain.	17.4149 g.
III Sheep Brain.	4.0020 g.
IV. Sheep Brain.	1[illegible] g.
V. Beef Brain.	[illegible].74[illegible] g.
VI. Sheep Brain.	11.[illegible] g.

Hydrolysis.

The preparations I, II, and III were hydrolysed with dilute hydrochloric acid (a 5% solution). The last three hydrolyses were carried out using aqueous potassium hydroxide (No. IV & No. V--[illegible] KOH) and (No. VI--1 [illegible] KOH). The hydrolysing mixture was boiled from 16 to 20 hours in a wide mouthed round-bottomed Jena flask with sufficiently large diameter to prevent any tendency toward bumping. This flask was connected to a water-cooled Hopkins reflux condenser. A slow stream of carbon dioxide was passed through the solution continuously during the hydrolysis period. This precaution was taken to prevent, if possible, oxidation of the unsaturated fatty acids. In case hydrolysis by potassium hydroxide had been used, the acids were liberated from their potassium salts by acidifying with dilute hydrochloric acid. When acid hydrolysis was used, the free acids floated on the surface when hydrolysis was completed. The acids were filtered out on a hardened filter and were washed on the filter with water con-

taining a trace of acetic acid.

Bases.

After removal of the fatty acids by filtration, the nitrogenous bases in form of their chlorides remained in the filtrate, together with the glycero-phosphoric acid. This solution was evaporated to dryness. In the earlier work this evaporation was done by distillation under diminished pressure; later the solutions were simply allowed to evaporate in a wide porcelain dish over the steam bath, using a low temperature. Two different methods were used for separation and identification of the bases. In lecithin samples I and II, the residue was taken up in the smallest amount of water and barium chloride solution added in excess, until a portion showed a precipitate of barium carbonate when saturated with CO_2. Ninety-five percent alcohol was added to precipitate the barium-glycerol-phosphate. This precipitate was filtered off and filtrate concentrated to dryness. The residue was taken up in alcohol and evaporated repeatedly to free it from alcohol insoluble material. Finally the residue was taken up once in absolute alcohol and evaporated. It was then dissolved in distilled water. To a volume measuring 150 c. c., were added 15 c. c., of concentrated H Cl and a solution of 15 grams of phosphotungstic acid dissolved in 30 c.c. of water. The solution was made up to 200 c. c.; it was warmed slightly, then allowed to cool in the refrigerator.

The choline phosphotungstate precipitate thus obtained was filtered out, and washed on the filter with 250 c. c., of a solution containing 3 grams of phosphotungstic acid and 5 c. c., of concentrated H Cl. To the filtrate, phenolphthalein was added and 50% sodium hydroxide was run in until alkalinity was reached. The solution was then diluted to 500 c. c., and a 20% solution of crystalline Ba Cl_2

added in excess. This removed the excess phosphotungstic acid. The precipitate was filtered out and washed free from chlorides. Washings and filtrate combined were acidified with H Cl and evaporated to dryness on steam bath. The residue was extracted with absolute alcohol.

The alcohol solution thus obtained was filtered and a warm saturated alcoholic solution of picrolonic acid
(1, p--nitrophenyl--3--methyl--4--nitro--5 pyrazolon)
was added. The picrolonate of amino-ethyl alcohol precipitated slowly on standing in a dessicator over sulphuric acid or Ca Cl_2. The crystals had the characteristic crystalline form described by Knorr(71). After being recrystallized several times from alcohol it began to soften at 221°C and melted with decomposition at 225°C. At this temperature it became entirely black. The salt prepared from synthetic amino ethyl alcohol showed exactly the same crystalline form and melting point. On mixing the synthetic with the natural derivative no change in melting point was observed.

It may be well to outline the procedure followed in the synthesis of the base. Knorr (71) first prepared the free base by repeating Wurtz's (72) work. This he did by action of ethylene oxide upon aqueous ammonia. The available starting point in our synthesis seemed to be ethylene bromide. Atkinson (73) prepared glycol acetate by refluxing on the water bath a mixture of ethylene bromide and potassium acetate with 80% alcohol. The portion distilling at 182°C was the glycol acetate.

According to Simpson (74), the hydrochloride of glycol acetate is prepared by saturation of glycol acetate with dry H Cl gas. The portion distilling over at 144-146° was glycol chloracetate. By action of strong KOH solution on this compound, ethylene oxide resulted.

We refluxed together for two days on the water bath a mixture of 175 grams potassium acetate and 175 grams ethylene bromide with 350 grams of 85% ethyl alcohol. We redistilled the fraction boiling from 150°-190°C and once more redistilled the fraction from 180°-185°. Most of it distilled at 182°C. This glycol acetate was saturated with dry H Cl gas generated by dropping dilute H_2SO_4 upon Na Cl, and dried by passing through H_2SO_4 and then through $Ca\ Cl_2$. The chloracetic ester separated as a brown oil. This oil was placed in a separatory funnel and dropped slowly into a 1:1 solution of concentrated KOH in water. The alkali was warmed slightly and the vapors passed through a condenser cooled by means of ice water drawn through by suction of a water pump.

The cooled vapors of ethylene oxide were then conducted into a flask containing a saturated solution of ammonia in ethyl alcohol, and surrounded by a freezing mixture. The flask was provided with a small outlet tube to release pressure. The alcohol solution, containing the ethylene oxide and ammonia, was allowed to stand tightly stoppered for two days. All tendency to explosion was eliminated by the careful cooling.

The solution was fractionated. At first ammonia vapor and alcohol passed over. Fractions from 85°-130° were redistilled. Fractions boiling at 120°-180° were also redistilled. Finally combining the higher boiling fractions all distilled over at 120°-140°C. This substance was a light brown oil which rapidly grew dark in color. It had a strong basic odor and attacked cork readily. Assuming the specific gravity to be slightly greater than one, the yield was approximately two grams.

The picrolonate derivative of the synthetic base proved to have the same properties as the derivative of the natural base, as

described previously. The melting or decomposition point was 225°C. The crystals form peculiar tufts of yellow needles, as Knorr says, with characteristic twinning. See cut.

The percent nitrogen determined in the picrolonate by Kjeldahl method was on two checks I 21.25% and II 21.20%, which agrees well with Knorr's value, i. e., 21.51%. The calculated value is 21.51%.

Lecithin samples III, IV, and V were examined together for amino ethyl alcohol. Sample VI was treated separately by the same method. Samples III-VI were analysed for bases by a different method from the one above outlined for samples I and II. The solution of chlorides of the bases were evaporated to dryness and extracted with absolute alcohol, then a solution of platinum chloride was added, to precipitate the choline. The precipitate came down in fine flocculent form. Recrystallised from dilute alcohol, it appeared as fine platelets of a yellow color. By ignition of a sample in a porcelain crucible, 31.6% platinum was obtained. Gulewitsch (75) obtained 31.7 and 31.8% platinum. Calculated percent platinum is 31.64%.

The excess platinum was removed from the alcoholic solution of the bases, by saturation with H_2S. The glycero-phosphoric acid

was not removed as was done in the first preparations, but potassium hydroxide in sticks was added to the strongly concentrated alcoholic solution in a distilling bulb, until the solution reacted alkaline. Amino ethyl alcohol was then distilled off, leaving a residue of potassium glycero-phosphate. The largest fraction distilled over at 160°-165°. The purest fraction distilled at 177°-185°.

We obtained the same substance (amino ethyl alcohol) as was obtained by the previous method and with much less trouble. The picrolonate and the chloraurate were prepared.

The chloraurate was made by adding concentrated H Cl to the base and then a molecular equivalent of gold chloride in a 5% aqueous solution. After standing a week in a good vacuum dessicator over sulfuric acid, the salt crystallised in long needles over a centimeter long, of a golden yellow color. This salt was so hygroscopic that it was difficult to obtain a melting point at all. It seems to form a rather definite hydrate which melts at 76°. By long heating in a glas tube immersed in sulfuric acid maintained at 120°-150°C for two hours the salt became free from water and melted at 186°C. This agrees with Knorr's value. On attempting to crystallise the salt from water solution it was found to be impossible. This was also the experience of Trier. It was necessary to recrystallise from concentrated H Cl. Metallic gold separated as the salt crystallised and it seemed impossible to get it free from precipitated gold. It was therefore necessary to determine the percent of gold present as metallic gold, and to deduct this value from the total percent of gold in the salt. This was done by use of an alundum filter cone.

After treatment with concentrated H Cl, washing with water, then with alcohol and ether, the cone was dried in a steam oven. It was then ignited to constant weight. Then a sample of the chloraurate

was weighed out in the cone, treated with concentrated HCl, washed with water, then with alcohol and ether, then dried and ignited as before. The free metallic gold was found to be 2.13%. The total gold was found by ignition of the chloraurate to be 51.31% and 51.5% respectively. By subtraction of the metallic gold, 49.16% and 49.37% gold were obtained. The salt obtained by crystallising the filtrate from the metallic gold in the alundum cone, showed a gold content, after ignition of 49.4%. Knorr obtained 49.15% and 49.02% gold. Trier's value was 49.27% gold. Calculated for $C_2H_7NO\text{--}HCl\text{--}AuCl_3$ gold equals 49.17%.

It seems evident from the boiling point of the free base and analyses of thechloraurate and picrolonate derivatives together with a comparison of the same with the synthetic preparations, that one of the nitrogen-containing bases of beef and brain lecithin is amino ethyl alcohol. That choline is also present was determined by analysis of the platinum salt as above described.

Tables Showing Analyses of Bases.

Choline

Choline platinum chloride.

Wt. sample	Wt. Pt.	% Pt.	Theoretical % Pt.	Gulewitsch value
.2784 grams	.0888	31.8	31.64	31.8%

Amino Ethyl Alcohol Derivatives.

Picrolonate

Melting Point	Knorr's M. P.	% N_2	Theoretical % N_2	Knorr's value. % N_2
221°-225°C	221°-225°C	21.25 21.20	21.51	21.51

Chloraurate

I Total % gold	II % Free metallic gold	III % Gold by difference (I-II)
51.51		49.18
51.5	2.15	49.37

IV % Gold after separation of metallic gold.	V Theoretical % gold
49.4	49.17

VI Knorr's value % gold	VII Trier's value % gold.
49.15	
49.02	49.27

Fatty Acids.

After being washed on the filter, the acids were dissolved in 5% KOH solution. The solution was made slightly acid with acetic acid, then exactly neutralized with KOH. The solid acids were then separated from the liquid acids by the usual Gusserow-Verantrapp method for preparation and separation of the lead soaps. The unsaturated acids were liberated from their lead soaps by shaking the ether solution of the latter with dilute hydrochloric acid (1:4). Iodine values of 72%-85%, determined on the unsaturated acids by Hübl's method, seemed to indicate chiefly oleic acid. Iodine values varied according to the state of oxidation and purity of the acids. Some of the best values were 80.9%, 85.8% and 84.56%.

To further separate and identify the unsaturated acids, they were brominated in ether solution, by addition of a calculated excess of bromine dissolved in glacial acetic acid. The reaction was carried out in a flask immersed in a freezing mixture, and the flask was then placed in a refrigerator over night. The acids which

form hexabromides or higher bromides will be thus precipitated as insoluble bromides.

An insoluble white precipitate was obtained, which seems to indicate that a trace of highly unsaturated acid is present. This bromide was obtained in amounts too small to make possible a bromine determination. The next morning the precipitate was centrifuged out and the excess bromine was removed from the ether solution by shaking with a saturated solution of sodium thiosulphate. The solution was then washed free from salts in a separatory funnel. The ether layer was removed and placed in flask and the ether evaporated in a stream of CO_2 on the steam bath.

Two different bromides were indicated by their solubilities in petroleum ether. The bulk of the bromide was a brown colored liquid, rather viscous, and readily soluble in petroleum ether. This was probably oleic bromide. A small amount of a white solid bromide remained insoluble. It was difficult to completely free the oleic bromide from this trace of insoluble matter, as the latter seems to be soluble in oleic bromide. It was not determined whether or not the insoluble portion was linolic bromide, as might have been indicated by its solubility. The amount of the material was too small for analysis. It may have been that linolic acid was present in the original lecithin only as an impurity. On allowing the oleic bromide to stand in a vacuum dessicator over H_2SO_4, the trace of insoluble matter (linolic bromide) crystallized out. The liquid oleic bromide was decanted after addition of a drop of petroleum ether to decrease the viscosity.

The percent of bromine was calculated in the oleic bromide; a sample was fused with KOH and KNO_3 in a nickel crucible, over a low flame. When all brown color had disappeared, the fusion mixture was

dissolved, acidified with nitric acid and the bromine determined by the Volhard method. We obtained a mean value of [illegible]9% bromine which agrees more closely with the bromide of oleic acid than with the other.

Both the bromine and the iodine values were somewhat too low for oleic acid in the pure condition. But if it be remembered that this acid is readily oxidized and that the separation of saturated from unsaturated acids is at best only incomplete, the results may well be explained. We conclude that one of the unsaturated acids of brain lecithin is oleic; and that either linolic and one other highly unsaturated acid are present in rather small amounts or as impurities in the original lecithin.

The saturated acids were obtained from the lead soaps by digestion with dilute hydrochloric acid. The acids were washed, separated from traces of unsaturated acids by cooling the concentrated acetone solution in a ice salt mixture and filtering out the saturated acids. They were then recrystallised from alcohol. Only in lecithin II were the saturated acids obtained in sufficiently pure condition for characterization. The saturated acid portion of this lecithin sample had a melting point of 65°C. and a molecular weight of 290. The melting point would indicate 65% stearic and 35% palmitic acid, but the material was not absolutely pure. The molecular weight would indicate only stearic acid. But this evidence is not conclusive for the molecular weight was determined on a very small sample. Only .0394 grams of acid were obtained of sufficient purity to be used in molecular weight determination. It seems highly probable, however, that the only saturated acid present is stearic. The ratio of saturated to unsaturated acids varied as is indicated by the following representative data:

Saturated		Unsaturated
1.4015 grams	:	1.1882 grams
1.4507 "	:	1.1823 "

Further work should be done on both the saturated and the unsaturated acids.

V. DISCUSSION OF RESULTS.

Lecithins from sheep brain and from beef heart in both contained amino ethyl alcohol. Choline was also present in both. Since a new constituent of lecithin has been discovered, it is of prime importance that the composition of any lecithin sample used in feeding experiments should be thoroughly determined before conclusions can safely be drawn from such experiments.

Confirmation of the presence of amino ethyl alcohol in lecithin is an important contribution toward the solution of the problem concerning the distribution of the amino nitrogen in the lecithin molecule. The nitrogen unaccounted for by choline may well be present in amino ethyl alcohol. Moreover, the impossibility of quantitative determination of lecithin on basis of choline content is strongly emphasized. Indeed it is still uncertain whether each lecithin molecule contains a molecule of choline and a molecule of amino ethyl alcohol. It may be that a lecithin molecule is a complex of two molecules of the usual size, containing choline in one and amino ethyl alcohol in the other.

According to Malengreau and Prigent (65), the splitting off of choline and of the fatty acids takes place at the same time and more rapidly than the phosphoric acid is freed from glycero-phosphoric acid. They think the linking of phosphoric acid to glycerine must be different from the linkage to choline. It is conceivable that the lin-

ferent linkage may be brought about by condensation of the amino group of amino ethyl alcohol with the double bonded oxygen of phosphoric acid. Choline might then be attached to amino ethyl alcohol by dehydration between two hydroxyl groups. This makes choline attached loosely to phosphoric acid through another base. The intermediate base would be attached to phosphorus by a double bond between nitrogen and phosphorus. The ease with which the basic part is split off would be thus explained. Compounds containing phosphorus doubly linked to nitrogen have not been found, (at least not to our knowledge), but it is reasonable to suppose that such compounds may exist.

A different mode of linkage may not be necessary to explain the difference in rate of hydrolysis, for it may depend solely upon the nature of the constituents and not upon the structure. No compound of amino ethyl alcohol and choline has been isolated. But since choline has been precipitated by chlorides of heavy metals, the complex may have been broken apart in the process of precipitation, if not already broken up in hydrolysis.

It also seems likely that the usual formula for lecithin is wrong in another respect, namely in representing a free hydroxyl group attached to phosphorus and a second one in choline. Dehydration of water would probably occur between these two groups.

Another idea suggested by discovery of amino ethyl alcohol in lecithin is that of Trier. He believes that a nitrogen-free lecithin first forms, and then a lecithin containing amino ethyl alcohol is produced by action of ammonia. The choline lecithin may, Trier suggests, be formed by methylation of the "colamine lecithin." Now it seems probable that lecithin may contain two lecithin molecules, one a "colamine lecithin" and another a "choline lecithin." The fatty acids in the two lecithins may be the same or they may contain differ-

ent unsaturated acids. The molecules may both contain oleic acid, or one molecule may contain other unsaturated acids like linolic and clupanodenic.

Obviously, more quantitative data on both acids and bases is necessary. A molecular weight determination of lecithin would be very valuable. Until more work is done, we would not be justified in trying to postulate a structural formula.

VI SUMMARY.

1. We have found by hydrolysis of brain lecithin (sheep and beef) and preparation and identification of the derivatives, that amino ethyl alcohol and choline are both present.

2. Since amino ethyl alcohol has been found by Trier, in egg and in bean and oat-grit lecithin; and since we have found it also in both beef and sheep brain lecithin, we may justly conclude that the majority of lecithins normally contain amino ethyl alcohol as one of the nitrogen-containing bases, and that those lecithins which do not contain this base are to be looked upon as exceptions.

3. Our work on fatty acids, though not conclusive, seems to indicate that oleic and stearic acids are present; possibly linolic and another highly unsaturated acid may be present in small amounts. This work serves to impress the necessity of further investigation of the fatty acids.

4. Quantitative data is needed both on the molecular weight of lecithin and the relative percents of choline, glycero-phosphoric acid and amino ethyl alcohol. Quantitative determination of the proportions of each constituent will aid greatly in assigning a definite formula for lecithin. We have only succeeded in definitely proving

that the present formula for lecithin is incorrect inasmuch as it does not include amino ethyl alcohol as a constituent.

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